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Monoolein-based cubosomes affect lipid profile in HeLa cells

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ABSTRACT

Monoolein-based cubosomes are promising drug delivery nanocarriers for theranostic purposes. Nevertheless, a small amount of research has been undertaken to investigate the impact of these biocompatible nanoparticles on cell lipid profile. The purpose of the present investigation was to explore changes in lipid components occurring in human carcinoma HeLa cells when exposed to short-term treatments (2 and 4 h) with monoolein-based cubosomes stabilized by Pluronic F108 (MO/PF108). A combination of TLC and reversed-phase HPLC with DAD and ELSD detection was performed to analyze cell total fatty acid profile and levels of phospholipids, free cholesterol, triacylglycerols, and cholesteryl esters. The treatments with MO/PF108 cubosomes, at non-cytotoxic concentration (83 μg/mL of MO), affected HeLa fatty acid profile, and a significant increase in the level of oleic acid 18:1 *n*-9 was observed in treated cells after lipid component saponification. Nanoparticle uptake modulated HeLa cell lipid composition, inducing a remarkable incorporation of oleic acid in the phospholipid and triacylglycerol fractions, whereas no changes were observed in the cellular levels of free cholesterol and cholesteryl oleate. Moreover, cell-based fluorescent measurements of intracellular membranes and lipid droplet content were assessed on cubosome-treated cells with an alternative technique using Nile red staining. A significant increase in the amount of the intracellular membranes and mostly in the cytoplasmic lipid droplets was detected, confirming that monoolein-based cubosome treatment influences the synthesis of intracellular membranes and accumulation of lipid droplets.

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1. Introduction

Nanoparticles (NPs) are a class of functional materials characterized by size-dependent properties, generally defined as engineered structures with at least one dimension less than 100 nm (Kroll et al., 2009), for which applications in medicine as therapeutic drug delivery and/or medical imaging systems have been predicted (Faraji and Wipf, 2009; Kroll et al., 2009; Panariti el al., 2012). Relevant examples include micelles, liposomes, solid lipid, polymeric, silicon-based, gold, or iron oxide NPs, as well as dendrimers, and quantum dots (Faraji and Wipf, 2009; Panariti el al., 2012). Lipid-based reverse cubic bicontinuous liquid crystalline phases possess a three-dimensional structure consisting of non-intersecting bilayers folded on an infinite periodic minimal surface characterized by a cubic symmetry and organized to form two disconnected, continuous water channels (Hyde, 1989). They were broadly investigated for pharmaceutical purposes in the past as their nanostructure can incorporate molecules of biological relevance (Caboi et al., 2001; Murgia et al., 2001). Remarkably, these peculiar cubic phases can be dispersed in water originating nanoparticles known as cubosomes (Larsson, 1983; Larsson and Tiberg, 2005), often pictured as the non-lamellar counterpart of liposomes. Cubosomes can be easily prepared sterically stabilizing a dispersion of monoolein (MO, Fig. 1A) in water by Pluronic series. By virtue of peculiar characteristics such as high mechanical rigidity, high hydrophobic volume, and the possibility of being biodegraded in vivo through enzyme-catalyzed reactions (carboxylesterases and phosphatases) (Hinton et al., 2014; Mulet et al., 2013), monoolein-based cubosomes were recently proposed for application in theranostic nanomedicine (Caltagirone et al., 2014; Murgia et al., 2013).

Several investigations were conducted on the in vitro cytotoxicity of MO-based nanoparticles, and results were found related to cell line, incubation time, dose, and formulation type (Falchi et al., 2015; Hinton et al., 2014; Murgia et al., 2010, 2015; Tran et al., 2015). Previous studies also provided evidence that, as result of their internalization, MO-based cubosomes induce accumulation of lipids in treated cells, causing the increase (both in size and number) of the cytoplasmic lipid droplets (LDs) (Caltagirone et al., 2014; Falchi et al., 2015; Murgia et al., 2015). LDs are

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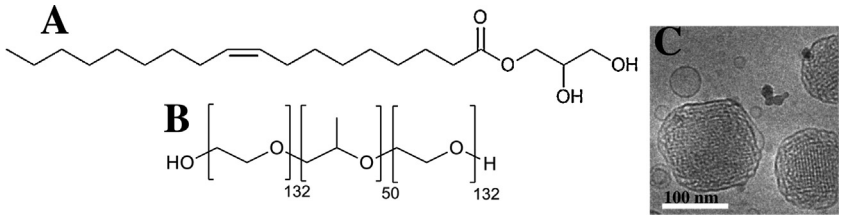

Fig. 1. Chemical structures of monoolein (MO) (A) and pluronic PF108 (B). Cryo-TEM images of MO/PF108 cubosomes (Falchi et al., 2015) (C).

macromolecular lipid assemblies consisting of neutral lipids, such as triacylglycerols, diacylglycerols, cholesterol esters, and cholesterol, surrounded by a monolayer of phospholipids and associated proteins (Bartz et al., 2007; Khatchadourian and Maysinger, 2009; Przybytkowski et al., 2009; Suzuki et al., 2012). LDs are now regarded as metabolically active organelles, with a particular structure and organization, engaged in a wide range of activities and formed, under physiological conditions, when free (unesterified) fatty acids from exogenous or endogenous sources are available inside the cells (Khatchadourian and Maysinger, 2009; Przybytkowski et al., 2009; Suzuki et al., 2012). Their main functions includes lipid storing and supplying for various cellular needs (β-oxidation, membrane biogenesis, and lipoprotein synthesis) (Suzuki et al., 2012).

Remarkably, although numerous articles were devoted to validate cubosome relevance in nanomedicine, only a small amount of research was undertaken to investigate the impact of these nanoparticles on the cell lipid profile (Falchi et al., 2015). The present investigation aimed to fill this gap by exploring the changes in lipid components occurring in HeLa cells when exposed to short-term treatments with MO-based cubosomes stabilized by Pluronic F108 (MO/PF108). The effect of nanoparticles (at 2 and 4 h of incubation) on HeLa cell viability was preliminary evaluated by the MTT assay. MO/PF108 cubosomes, at non-cytotoxic concentration, were then tested to evaluate their effect on lipid component profile, with particular regard to triacylglycerols (TAG), phospholipids (PL), free cholesterol (FC), cholesteryl esters (CE), and total fatty acid composition. In situ fluorescent quantification of cytoplasmic membranes and LDs after cubosome treatment was also assessed in living cells loaded with Nile red, a fluorescent hydrophobic probe for the detection of polar and non-polar lipids (Greenspan et al., 1985).

2. Materials and methods

2.1. Materials

Monoolein (RYLO MG 19 PHARMA, glycerol monooleate, 98.1 wt %; MO) was kindly provided by Danisco A/S, DK-7200, Grinsted, Denmark. Pluronic F108 (PEO_{132}–PPO_{50}–PEO_{132}) (Fig. 1B), cholesterol, cholesteryl oleate (CO), cholesteryl arachidonate, standards of fatty acids, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (PC 16:0/16:0), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (PC 18:1/18:1), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (PC 16:0/18:1), 1-oleoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (PC 18:1/16:0), 2-linoleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine (PC 16:0/18:2), 2-arachidonoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine (PC 16:0/20:4), 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (PC 18:2/18:2), 1,2-dieicosapentaenoyl-*sn*-glycero-3-phosphocholine (PC 20:5/20:5), trilinolein (LLL), triolein (OOO), 1,3-dioleoyl-2-palmitoyl-glycerol (OOP), trilinolenin (LnLnLn), 1,2-dilinoleoyl-3-palmitoyl-rac-glycerol (LLP), 1,2-dilinoleoyl-3-oleoylrac-glycerol (LLO), 1,2-dioleoyl-3-linoleoy-rac-glycerol (OOL), and Desferal (deferoxamine mesylate salt), were purchased from Sigma–Aldrich (Milan, Italy). The code letters used for the fatty acid in TAG are: L, linoleic; Ln, linolenic; O, oleic; P, palmitic. All solvents used, of the highest available purity, were also from Sigma–Aldrich. All of the other chemicals used in this study were of analytical grade. Cell culture materials were purchased from Invitrogen (Milan, Italy).

2.2. Cubosome preparation and characterization

Cubosomes were prepared by dispersing the appropriate amount of melted MO in a solution of Pluronic F108 using an ultrasonic processor UP100H by Dr. Hielscher, cycle 0.9, amplitude 90%, for 10 min. The sample volume was 4 mL with 96.4 wt% of water, 3.3 wt% of MO and 0.3 wt% of Pluronic F108 (Falchi et al., 2015). Distilled water passed through a Milli-Q water purification system (Millipore) was used to prepare the samples. MO/PF108 cubosomes (Fig. 1C) were characterized for particle size, morphology, and inner structure as previously reported (Falchi et al., 2015).

2.3. Cell Culture

Human carcinoma HeLa cell line (ATCC collection) was grown in phenol red-free Dulbecco's modified Eagle's medium (DMEM, Invitrogen, USA) with high glucose, supplemented with 10% (v/v) fetal bovine serum, penicillin (100 U mL^{-1}), and streptomycin (100 μg mL^{-1}) (Invitrogen) in a 5% CO_2 incubator at 37 °C.

2.4. Cytotoxic activity (MTT assay)

The cytotoxic effect of the nanoparticle formulation MO/PF108 was evaluated in HeLa cells by the MTT assay (Rosa et al., 2013; Schiller et al., 1992). HeLa cells were seeded in 24-well plates at density of 3×10^4 cells/well in 500 μL of serum-containing media. Experiments were carried out two days after seeding when cells had reached 90% confluence. Cubosomes were added to the cells at concentrations of 1:400 and 1:200 (2.5 μL and 5 μL in 1 mL of serum-free medium, respectively) and incubated at 37 °C for 2 h and 4 h. A 50 μL portion of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (5 mg/mL in H_2O) was then added and left for 2 h at 37 °C. The medium was aspirated, 500 μL of DMSO was added to the wells, and colour development was measured at 570 nm with an Infinite 200 auto microplate reader (Infinite 200, Tecan, Austria). The absorbance is proportional to the number of viable cells. All measurements were performed in quadruplicate and repeated at least three times. Results are shown as percent of cell viability in comparison with non-treated control cells.

2.5. Lipid profile modulation in HeLa cells

HeLa cells were plated in Petri dishes at a density of about 10^6 cells/10 mL of complete medium and were used for fatty acid profile modulation experiments at 2 days post-seeding when cells

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